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BACTERIA IN ICE CREAM---II

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BACTERIA IN ICE CREAM--II

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The work done in various laboratories during the last 10 to 15 years has clearly established that the ice cream ordinarily offered for sale contains large and frequently enormous numbers of bacteria. As a result, certain health departments have begun to consider the sanitary as well as the chemical condition of the ice cream sold within their districts and have established various regulations intended to prevent the sale of a product that may contain organisms liable to set up diseased conditions in the persons consuming it. These regulations are undoubtedly of value and many of them have been of great help in improving the quality of the ice cream sold, but it is very evident to persons giving them any attention that our knowledge of the bacteria in ice cream, as well as the influence of such procedures as storage, freezing and hardening on the numbers of organisms present, is insufficient to permit of the establishment of fair and consistent bacterial standards.

The dairy section of the Iowa Agricultural Experiment station [1] has already reported some work on the bacteriology of ice cream which shows that, unless cream is pasteurized, it is ordinarily the most important source of the bacteria in ice cream, that gelatin may carry from very few to enormous numbers of bacteria, that vanilla is of no importance as a source of bacteria, that sugar properly cared for is of little consequence as a source of contamination and that the bacteria which develop on agar at 37° C. do not increase in ice cream kept suitably hardened. It was also suggested that the utensils undoubtedly may be important sources of contamination here as with other dairy products if they are not properly cared for. The data secured also show that ice cream with a very low bacterial count may be manufactured by the employment of methods which are perfectly practical.

Ayers & Johnson [2] studied the numbers of bacteria in 94 samples of ice cream secured during the summer and in 91 samples secured during the winter, all taken from ice cream on sale at retail in Washington. Seventy-one summer samples and 28 winter samples were studied by the milk-tube method in order to determine the percentage of the various groups present.

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1 B. W. Hammer, Bacteria and Ice Cream. Bull. Ia. Agr. Expt. Sta. 134. July, 1912.

2 A Bacteriological Study of Retail Ice Cream. Bull. U. S. Dept. of Agr. 303. Oct., 1915.

Esten & Mason [3] concluded from a study of 12 samples that, "When ice cream is kept frozen for periods of at least a month there is no marked increase or decrease in the bacteria content as shown by litmus lactose gelatin plate cultures. The percentage of acid bacteria and of liquifying bacteria also remained fairly constant."

SCOPE OF INVESTIGATION HERE REPORTED

In the work reported in the present paper the following points have been taken up:

a. Contamination from the freezer.
b. Numbers of bacteria in water sherbets.
c. Numbers of bacteria in ice creams other than vanilla.
d. Changes in numbers during the storage of ice cream.
e. Influence of freezing* on the bacterial count.
f. Influence of hardening* on the bacterial count.
g. Influence of softening and rehardening on the bacterial count.

Only the numbers of bacteria have been considered in dealing with these various points; no work has been done on the types of bacteria or on the changes in types because it is believed that until the types of organisms contained are more widely considered in bacterial standards for milk there is no justification for considering them in bacterial standards for ice cream.

METHODS

All determinations of the numbers of bacteria were made on the c.c. basis instead of on the gm. basis because of the great saving in time. Samples taken from containers were secured by means of a butter trier that had been sterilized and allowed to cool to room temperature; after the core was withdrawn it was cut in two over the edge of a sterile Petri dish and the upper part discarded so as to eliminate the ice cream from the surface of the container. Samples from the freezers were allowed to run directly into sterile Petri dishes. Both classes of samples were slowly warmed over a Bunsen burner until the entire amount was melted and were then well mixed by rotating the Petri dish. A c.c. portion was withdrawn and treated exactly the same as a

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3 Bacteriological Studies: The Bacterial Content of Ice Cream. Bull. Storrs Agr. Expt. Sta. 83. S. 1915.

* The terms "freezing" and "hardening" will be explained because, where ice cream is made in considerable amounts, methods are commonly employed which are different from those apparently used by certain investigators. After "freezing" the ice cream is still so soft that it runs from the freezer and can be readily poured; the temperature is ordinarily from 26 to 28° F. The frozen ice cream is hardened by being packed in ice and salt or by having held in a low temperature room; properly hardened ice cream is rarely above 15° F. and usually is under 10° F. It is evident that the conditions prevailing in hardened ice cream are quite different from those in ice cream that has been simply frozen and the former would ordinarily be assumed to be much more destructive to bacteria.

c.c. of milk in the determination of the number of bacteria therein.

The plating medium used for the ice cream was agar prepared from meat infusion as formerly recommended for milk analysis by the American Public Health Assn.; the same medium was used for sherbet because it seems inadvisable to use different media on products so similar as sherbet and ice cream. The incubation temperature was 37° C. and the time forty-eight hours. The results presented are the average of two plates except in a few cases where one plate was lost thru spreaders.

RESULTS OBTAINED

(a) *Contamination from the freezer*

In a previous publication of this station,[4] the possibility of the utensils used being a source of bacterial contamination was pointed out. In order to determine the importance of this source of contamination, sterile water was added to a freezer and the machine operated for 10 minutes (approximately the freezing period with the machine used) after which the number of bacteria per c.c. of water was determined. The results secured are presented in table I.

TABLE I. CONTAMINATION FROM ICE CREAM FREEZER

Bacteria per c. c. of Water after Ten Minutes Agitation in Freezer

Trial number	Pounds of water	Bacteria per c.c. of water used	Time of washing
1	71	3,700	Washed day previous to test
2	74.5	141,500	Washed day previous to test
3	73.5	8,050	Washed day previous to test
4	72.0	1,195	Washed 2 days previous to test
5	71.5	300	Washed day previous to test

The counts secured in the five trials range from 300 to 141,500 per c.c. of water added. Because of the failure to secure an increase in the volume of the water, due to the fact that the water would not hold the air beaten into it, it was necessary, in order to fill the machine, to use a larger volume of water than is ordinarily used of an ice cream mix and accordingly the increase per c.c. of water was considerably less than would be true in the case of ice cream; the increase per c.c. of an ice cream mix would have been fully twice the increase recorded for water in table I and perhaps more. From these data it is evident that the freezer may be of importance as a means of contamination where an effort is being made to produce ice cream with a low bacterial count, and that the cleaning of the freezer must be carefully done. If water is left in the freezing chamber after the washing, there is very likely to be a considerable multiplication of the

[4] See Ref. 1.

remaining bacteria and, consequently, drying after washing is as important here as with other dairy utensils. Undoubtedly it would be advantageous to sterilize* the freezing chamber just before using the freezer. In this connection, the results obtained during the freezing of a batch of low count ice cream are rather suggestive; the mix contained 6,450 bacteria per c.c., the first batch of ice cream 20,150 and the second batch 6,800. The much higher count secured on the first batch than on the second was very probably due to the contamination from the freezer.

(b) *Numbers of bacteria in water sherbets*

Seventeen samples of water sherbet were examined for the numbers of bacteria contained and the results secured are presented in table II.

TABLE II. BACTERIAL CONTENT OF WATER SHERBETS

Flavor	Bacteria per c.c.
Orange	3,275
Lemon	6
Lemon	6,540
Lemon	80
Cranberry	48
Cranberry	3,645
Mint	7,800
Cherry	115
Cherry	145
Grape	740
Grape	1,260
Raspberry	2,350
Pineapple	6,600
Pineapple	700
Pineapple	750
Pineapple	900
Pineapple	1,100

The counts range from 6 to 7,800 per c.c. There seems to be no relationship between the flavor of the sherbet and the bacteria per c.c.; one sample of cranberry sherbet contained 48 and another 3,645 bacteria per c.c., the counts on three samples of lemon sherbet varied from 6 to 6,540 per c.c., while the counts on five samples of pineapple sherbet varied from 700 to 6,600 per c.c.

The counts given were secured over a period of two years. As compared with the counts ordinarily secured on ice cream, water sherbets contain very few organisms. This substantiates the statement already made by this station that the cream is the main source of the bacteria in ice cream, since the absence of cream or milk in water sherbet is one of the important differences between this product and ice cream. Undoubtedly the acid present in sherbet as one of the results of the use of considerable quantities of fruit juices is responsible for the destruction of some of the bacteria, but it seems that the important factor causing the low bacterial count in water sherbets must be the absence of milk or milk derivatives. The amount of acid in sherbets is commonly sufficient to cause a clouding of the agar when the melted and cooled medium is mixed with the sherbet to be plated, and this makes the plating of sherbets somewhat

* If the freezing chamber is heated with steam or hot water, it must be cooled slowly by circulating warm water whose temperature is gradually reduced. If cold brine is circulated, the freezer is likely to be damaged by the sudden change in temperature.

more difficult that the plating of ice cream. The acid is also probably responsible for the small size of the colonies in some cases and it may even prevent the development of certain bacteria into colonies.

(c) *Numbers of bacteria in ice creams other than vanilla*

The number of bacteria present in ice creams other than vanilla has been reported in a few samples by various laboratories. In order to include a range of types of ice creams the data presented in table III, which deal with thirteen sample of ice creams, were collected. The counts range from 130,000 to 40,850,000 per c.c. and indicate that ice creams other than vanilla, like vanilla ice cream, ordinarily contain large numbers of bacteria. Altho the counts are too few in number to admit of any definite conclusions, there seems to be no relationship between the number of bacteria per c.c. and the type of ice cream and it is probable that the cream is, under ordinary conditions, the principal source of the bacteria in ice creams other than vanilla in the same way that it is in ordinary ice cream.

TABLE III. BACTERIAL CONTENT OF ICE CREAMS OTHER THAN VANILLA

Kind of Ice Cream	Bacteria per c.c.
Pudding, Nesselrode	740,000
Pudding, Manhattan	130,000
Pudding, Manhattan	4,200,000
Parfait, Chestnut	1,105,000
Parfait, Golden	1,760,000
Parfait, Golden	40,850,000
Bisque	17,000,000
Bisque	360,000
Ice Cream, Raspberry	150,000
Ice Cream, Caramel	740,000
Ice Cream, Maple Nut	650,000
Ice Cream, Chocolate	1,440,000
Ice Cream, Chocolate	350,000

(d) *Changes in numbers during the storage of ice cream*

The necessity of having definite information regarding the influence of storage on the bacterial content of ice cream has already been pointed out by this station and certain data on this point, which were collected on ice cream held packed with ice and salt, have been presented. Because of the importance of the point, however, fifty-one additional samples of ice cream have been studied from the standpoint of the influence of storage on the bacterial content and the data obtained are presented in tables IV to LIV inclusive. Thirty-nine of the samples (tables IV to LIV inclusive) were held by packing in ice and salt while twelve (tables XLIII to LIV inclusive) were held in a commercial hardening room. The larger number of samples were held with ice and salt because it is probable that ice cream held in this manner reaches higher temperatures than ice cream held in hardening rooms, and accordingly would undoubtedly be more likely to show increases in the number of organisms.

There is no evidence in the results presented that there is ever

an increase in ice cream held under proper conditions. Altho in a good many instances there are slight increases in the recorded counts with an increase in the period of holding, these increases are so small that they must be considered within the limit of experimental error; this is especially true since ice cream is a material with which, because of the nature of the product, rather wide variations in the results of bacterial counts are to be expected. A considerable number of the samples show undoubted decreases in the numbers of organisms which is presumably the result of the dying off of some of the bacteria as a consequence of the low temperatures. In general then, during the proper storage of ice cream, there is no increase in the number of organisms present and there is very likely to be a decrease.

The results obtained on the material held in the hardening room are essentially the same as those secured on the ice cream packed with ice and salt. While no difference was expected in the results secured with the two methods of holding, it seemed desirable to hold some of the samples under the conditions existing in the large plants.

TABULATIONS OF BACTERIAL COUNT ON 51 SAMPLES OF STORED ICE CREAM

TABLE IV. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	236,500
Freeze	735,000
1 day old	360,000
2 days old	310,000
3 days old	260,000
5 days old	310,000

TABLE V. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	9,000,000
Freeze	9,000,000
1 day old	7,250,000
3 days old	3,425,000
5 days old	2,170,000
7 days old	2,715,000

TABLE VI. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	1,475,000
Freeze	1,450,000
1 day old	760,000
2 days old	900,000
3 days old	850,000
4 days old	1,170,000
5 days old	415,000
7 days old	470,000

TABLE VII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	32,800,000
Freeze	30,850,000
1 day old	7,750,000
2 days old	4,450,000
3 days old	2,435,000
4 days old	1,150,000

TABLE VIII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	120,000
Freeze	146,500
1 day old	137,500
2 days old	216,000
4 days old	152,000
5 days old	300,000
6 days old	139,000
7 days old	156,500

TABLE IX. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	3,150,000
Freeze	8,750,000
2 days old	4,900,000
3 days old	8,650,000
4 days old	1,410,000
5 days old	2,400,000
6 days old	1,170,000

TABLE X. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	224,000
Freeze	224,000
1 day old	240,000
2 days old	199,500
4 days old	211,000
5 days old	182,000
7 days old	162,000
9 days old	149,000

TABLE XI. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	258,000
Freeze	268,500
1 day old	246,000
2 days old	219,000
4 days old	210,000
5 days old	142,500
7 days old	171,500
9 days old	131,000
11 days old	150,000

TABLE XII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	10,250,000
Freeze	18,850,000
1 day old	8,700,000
2 days old	8,950,000
4 days old	2,750,000
5 days old	2,300,000
7 days old	1,240,000
9 days old	1,400,000

TABLE XIII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	12,300,000
Freeze	18,300,000
1 day old	6,300,000
2 days old	7,000,000
4 days old	3,100,000
5 days old	2,300,000
7 days old	940,000
9 days old	1,400,000
11 days old	1,000,000

TABLE XIV. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	3,100,000
Freeze	4,750,000
1 day old	3,300,000
2 days old	3,150,000
3 days old	2,450,000
5 days old	1,850,000
7 days old	2,150,000

TABLE XV. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	3,150,000
Freeze	5,000,000
1 day old	2,600,000
2 days old	2,550,000
3 days old	2,050,000
5 days old	1,750,000
7 days old	1,875,000

TABLE XVI. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	5,850,000
Freeze	6,450,000
1 day old	4,950,000
2 days old	3,950,000
3 days old	4,150,000
7 days old	3,300,000
9 days old	1,500,000

TABLE XVII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	5,850,000
Freeze	8,550,000
1 day old	3,450,000
2 days old	3,300,000
3 days old	3,150,000
5 days old	3,000,000
7 days old	2,650,000

TABLE XVIII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	4,600,000
Freeze	5,150,000
1 day old	1,450,000
2 days old	690,000
4 days old	670,000
6 days old	1,160,000

TABLE XIX. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	5,300,000
Freeze	5,900,000
1 day old	1,900,000
2 days old	690,000
4 days old	595,000
6 days old	535,000
8 days old	295,000

TABLE XX. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	4,400,000
Freeze	5,500,000
1 day old	1,850,000
2 days old	635,000
3 days old	860,000
5 days old	865,000
7 days old	800,000

TABLE XXI. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	4,300,000
Freeze	5,400,000
1 day old	1,800,000
2 days old	680,000
3 days old	800,000
5 days old	955,000

TABLE XXII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	27,500
Freeze	45,500
1 day old	22,500
2 days old	22,500
3 days old	33,500
5 days old	23,000
7 days old	34,000

TABLE XXIII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	27,000
Freeze	44,000
1 day old	34,000
2 days old	28,000
3 days old	35,000
5 days old	32,000
7 days old	40,000

TABLE XXIV. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	34,000
Freeze	47,500
1 day old	35,500
2 days old	40,000
3 days old	28,000
5 days old	35,000
7 days old	35,500

TABLE XXV. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	36,500
Freeze	53,000
1 day old	31,000
2 days old	41,500
3 days old	34,000
5 days old	40,000
7 days old	42,500

TABLE XXVI. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	165,000,000
Freeze	240,000,000
1 day old	146,500,000
2 days old	120,500,000
3 days old	57,500,000
5 days old	30,500,000
7 days old	30,500,000

TABLE XXVII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	152,500,000
Freeze	227,500,000
1 day old	154,000,000
2 days old	149,000,000
3 days old	64,000,000
5 days old	25,500,000
7 days old	23,000,000

TABLE XXVIII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	172,500,000
Freeze	271,000,000
1 day old	157,500,000
2 days old	128,500,000
3 days old	52,000,000
5 days old	34,000,000
7 days old	31,000,000

TABLE XXIX. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	167,500,000
Freeze	285,000,000
1 day old	190,000,000
2 days old	153,500,000
3 days old	48,500,000
5 days old	28,500,000
7 days old	28,500,000

TABLE XXX. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	5,600,000
Freeze	4,700,000
1 day old	5,200,000
2 days old	4,200,000
4 days old	2,550,000
6 days old	2,300,000

TABLE XXXI. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	4,100,000
Freeze	3,900,000
1 day old	3,900,000
2 days old	4,250,000
4 days old	3,450,000
6 days old	2,200,000

TABLE XXXII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	320,000
Freeze	475,000
1 day old	465,000
4 days old	257,000
6 days old	360,000

TABLE XXXIII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	280,000
Freeze	380,000
1 day old	420,000
4 days old	315,000
6 days old	350,000

TABLE XXXIV. BACTERIA CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	400,000
Freeze	330,000
1 day old	310,000
2 days old	345,000
6 days old	320,000

TABLE XXXV. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	520,000
Freeze	510,000
1 day old	485,000
2 days old	310,000
4 days old	520,000
6 days old	305,000

TABLE XXXVI. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c
Mix	485,000
Freeze	660,000
1 day old	780,000
2 days old	385,000
4 days old	455,000
6 days old	470,000

TABLE XXXVII. BACTERIA CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	30,000
Freeze	33,500
1 day old	21,000
2 days old	16,500
4 days old	18,000
6 days old	26,000

TABLE XXXVIII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	28,500
Freeze	30,500
1 day old	30,000
2 days old	16,500
4 days old	23,000
6 days old	24,000

TABLE XXXIX. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	270,000
Freeze	285,000
1 day old	200,000
2 days old	160,000
4 days old	160,000
6 days old	170,000

TABLE XL. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	255,000
Freeze	370,000
1 day old	270,000
2 days old	190,000
4 days old	150,000
6 days old	175,000

TABLE XLI. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	2,220,000
Freeze	2,270,000
1 day old	1,660,000
2 days old	1,340,000
4 days old	1,940,000
6 days old	1,500,000
7 days old	1,100,000

TABLE XLII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	1,965,000
Freeze	2,120,000
1 day old	1,105,000
2 days old	1,105,000
4 days old	1,155,000
7 days old	1,107,000

TABLE XLIII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c.c.
Mix	3,410,000
Freeze	4,360,000
1 day old	2,765,000
2 days old	2,380,000
3 days old	2,115,000
4 days old	2,150,000
6 days old	2,900,000
8 days old	2,650,000
11 days old	2,025,000

TABLE XLIV. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	3,380,000
Freeze	3,875,000
1 day old	2,070,000
2 days old	3,320,000
3 days old	2,895,000
5 days old	2,240,000
7 days old	2,635,000
9 days old	2,215,000
11 days old	1,860,000

TABLE XLV. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	110,000,000
Freeze	145,000,000
1 day old	176,500,000
2 days old	218,500,000
3 days old	100,500,000
5 days old	25,000,000
7 days old	38,000,000
9 days old	26,500,000
12 days old	17,000,000

TABLE XLVI. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	110,000,000
Freeze	170,000,000
1 day old	194,500,000
2 days old	216,000,000
3 days old	102,000,000
5 days old	39,500,000
7 days old	54,000,000
9 days old	36,000,000
12 days old	15,500,000

TABLE XLVII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	181,500,000
Freeze	218,000,000
1 day old	145,000,000
2 days old	127,000,000
3 days old	62,500,000
5 days old	67,500,000
7 days old	62,500,000
10 days old	39,500,000

TABLE XLVIII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	1,310,000
Freeze	1,630,000
1 day old	1,350,000
2 days old	1,205,000
3 days old	1,500,000
5 days old	1,190,000
10 days old	775,000

TABLE XLIX. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	435,000
Freeze	300,000
1 day old	200,000
2 days old	200,000
4 days old	260,000
6 days old	170,000
9 days old	190,000

TABLE L. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	325,000
Freeze	115,000
1 day old	200,000
2 days old	475,000
4 days old	260,000
6 days old	190,000
9 days old	145,000

TABLE LI. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	435,000
Freeze	640,000
1 day old	341,000
3 days old	353,500
5 days old	306,000
8 days old	257,000

TABLE LII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	285,000
Freeze	495,000
1 day old	390,000
3 days old	315,000
5 days old	325,000
8 days old	280,000

TABLE LIII. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	21,000,000
Freeze	28,900,000
1 day old	12,300,000
3 days old	7,900,000
5 days old	6,950,000
8 days old	4,900,000

TABLE LIV. BACTERIAL CONTENT OF ICE CREAM DURING STORAGE

	Bacteria per c. c.
Mix	120,000,000
Freeze	140,000,000
1 day old	70,500,000
3 days old	71,500,000
5 days old	41,000,000
8 days old	61,000,000

(e) *Influence of freezing on the bacterial count*

From the data presented in tables IV to LIV inclusive, it is evident that significant changes occur in the numbers of bacteria in ice cream, as determined by the plate method, during the freezing process. The influence of the freezing can be determined by a comparison of the bacterial content of the mix and the frozen, but unhardened, ice cream. Such a comparison is influenced to a certain extent by the contamination from the freezer but, where the freezer is properly cared for, this contamination is of only little importance on a percentage basis with ice cream containing the ordinary number of organisms.

The influence of the freezing process on the bacterial content, as determined by the plate method, is shown in table LV; the majority of the results presented are retabulated from tables IV to LIV inclusive.

Of the 51 comparisons made, the frozen but unhardened ice cream had the same bacterial count (determined by the plate method) as the mix in two cases (4.0%), in six cases (11.8%) it had a lower count, the decrease varying from 2 to 31% and averaging 13.0% while in forty-three cases (84.3%) it had a higher count, the increase varying from 2 to 227% and averaging 46.3%.

From these results it is evident that there is usually an in-

TABLE LV. INFLUENCE OF FREEZING ON THE BACTERIAL CONTENT OF ICE CREAM

Bacteria per c. c.			Bacteria per c. c.		
Mix	Frozen material	% change in numbers	Mix	Frozen material	% change in numbers
150,000	490,000	227%	255,000	370,000	45%
19,100,000	24,750,000	30%	236,500	735,000	211%
2,220,000	2,270,000	2%	9,000,000	9,000,000	0%
1,965,000	2,120,000	8%	1,475,000	1,450,000	— 2%
3,410,000	4,360,000	28%	32,800,000	30,850,000	— 6%
3,380,000	3,875,000	15%	120,000	146,500	22%
110,000,000	145,000,000	32%	3,150,000	8,750,000	178%
181,500,000	217,000,000	20%	224,000	221,000	0%
1,310,000	1,630,000	24%	10,250,000	18,550,000	81%
435,000	300,000	—31%	3,100,000	4,750,000	53%
325,000	415,000	28%	12,300,000	18,300,000	49%
435,000	640,000	47%	3,150,000	5,000,000	59%
285,000	495,000	74%	5,850,000	6,450,000	10%
21,000,000	28,900,000	38%	4,600,000	5,150,000	12%
120,000,000	140,000,000	17%	5,300,000	5,900,000	11%
2,445,000	3,240,000	33%	4,400,000	5,500,000	25%
5,600,000	4,700,000	—16%	4,300,000	5,400,000	26%
4,100,000	3,900,000	— 5%	27,500	45,500	65%
320,000	475,000	48%	27,000	44,000	63%
280,000	380,000	36%	34,000	47,500	40%
400,000	330,000	—18%	36,500	53,000	45%
520,000	540,000	4%	165,000,000	240,000,000	45%
485,000	660,000	36%	152,500,000	227,500,000	49%
30,000	33,500	12%	172,500,000	271,000,000	57%
28,500	30,500	7%	167,500,000	285,000,000	70%
270,000	285,000	6%			

crease in the number of organisms, as determined by the plate method, during the freezing process. The big factor in accounting for changes in the numbers of organisms during the freezing process is the breaking up of clumps of organisms as a result of the agitation in the freezer. The breaking up of the clumps of organisms is of importance in increasing the bacterial content (as determined by the plate method) of milk passing thru certain types of clarifiers and this increase is properly considered as an apparent rather than a true increase.[5] With an ice cream mix in a freezer there is essentially the same situation; when a clump, which would ordinarily give rise to only one colony when the material is plated, is broken up, a number of colonies develop on plating. Another factor which, in all probability, plays a part in changing the bacterial content of a mix during the freezing process is the reduction in temperature, and this would be expected to cause the destruction of certain of the organisms. From the results presented in table LV, it is evident that the increase due to the agitation of the material is much more important than the decrease due to the reduction in temperature and ordinarily overshadows the later influence. It would seem, however, that with certain mixes in which there were few or no clumps that the temperature factor might be the more important and in accordance with this idea, table LV presents some data which show a smaller count on the frozen material than on the mix. The results obtained with certain clarifiers would suggest that the majority of the mixes would contain clumps enough so that there would be an apparent increase during the rapid agitation, and the results presented are in agreement with this.

Table LVI shows the bacterial counts secured on six ice cream mixes and on various lots of frozen ice cream made from each mix. Undoubtedly some of the variations in the results obtained on the frozen ice cream from the same mix are due to experimental error but it seems reasonable to suppose that the variation is in part due also to variations in the time and rate of agitation.

TABLE LVI. BACTERIAL COUNTS, PER C. C., SECURED ON ICE CREAM MIXES AND ON THE BATCHES OF ICE CREAM FROZEN FROM EACH

Mix	Material from Freezer				
	Batch A	Batch B	Batch C	Batch D	Batch E
6,450	6,800	9,300	8,350	9,000	
142,500	210,000	276,000	187,000	222,000	296,000
2,550	1,250	2,050			
325,500	402,000	437,000			
123,500	122,500	131,000	125,500	105,500	
56,500	79,000	65,500			

5 B. W. Hammer, Studies on the Clarification of Milk. Res. Bull. Ia. Agr. Expt. Sta. 28. Ja. 1916.

(f) *Influence of hardening on the bacterial count*

Another operation that has an important influence on the bacterial content of ice cream is the hardening process. Table LVII gives 52 comparisons of the bacterial content of frozen ice cream and ice cream after it has been hardened, the hardening generally being accomplished by holding over night packed in ice and salt. The most of the data are retabulated from tables IV to LIV inclusive.

Of the 52 comparisons made, the hardened ice cream showed the same bacterial count (determined by the plate method) as the unfrozen material in one case (2%), in 45 cases (86.5%) there was a decrease during the hardening process varying from 2 to 75% and averaging 39.1%, while in six cases (11.5%) there was an increase varying from 7 to 22% and averaging 13.8%.

The decreases in the 86.5% of the cases were undoubtedly due to the lowering of the temperature and the variations in the extent of the decreases were presumably due to variations in the resistance of the contained bacteria to the lowered temperatures. The temperature of the ice cream as it came from the freezer was from 26 to 28° F. while the temperature of the hardened ice cream was from 5 to 15° F., a drop of from 11 to 23° F. The increases in 11.5% of the cases were never large on a percentage basis, the maximum increase being 22%, and it seems

TABLE LVII. INFLUENCE OF HARDENING ON THE BACTERIAL CONTENT OF ICE CREAM

Bacteria per c. c.			Bacteria per c. c.		
Frozen material	Hardened material	% change in numbers	Frozen material	Hardened material	% change in numbers
2,270,000	1,660,000	27%	9,000,000	7,250,000	—19%
2,120,000	1,105,000	—48%	1,450,000	640,000	—56%
4,360,000	2,765,000	37%	30,850,000	7,750,000	—75%
2,875,000	2,070,000	—17%	146,500	137,500	— 6%
145,000,000	176,500,000	22%	8,750,000	4,900,000	44%
170,000,000	194,500,000	14%	21,000,000	5,320,000	—75%
218,000,000	145,000,000	—33%	224,000	240,000	7%
1,630,000	1,350,000	17%	268,500	246,000	— 8%
300,000	200,000	—33%	18,850,000	8,700,000	—54%
415,000	200,000	—52%	18,300,000	6,300,000	—66%
640,000	341,000	—47%	4,750,000	3,300,000	—31%
495,000	390,000	—21%	5,000,000	2,600,000	48%
28,900,000	12,300,000	—57%	6,450,000	4,950,000	—23%
140,000,000	70,500,000	—50%	8,550,000	3,450,000	60%
4,700,000	5,200,000	11%	5,150,000	1,450,000	—72%
3,900,000	3,900,000	0%	5,900,000	1,900,000	68%
475,000	455,000	— 4%	5,500,000	1,850,000	—66%
380,000	420,000	11%	5,400,000	1,800,000	—67%
330,000	310,000	— 6%	45,500	22,500	51%
540,000	485,000	10%	44,000	34,000	—23%
660,000	780,000	18%	47,500	35,500	—25%
33,500	21,000	—37%	53,000	31,000	42%
30,500	30,000	— 2%	240,000,000	146,500,000	—39%
285,000	200,000	—30%	227,500,000	154,000,000	—32%
370,000	270,000	—27%	271,000,000	157,500,000	42%
735,000	360,000	—51%	285,000,000	190,000,000	—33%

that they must be the result of experimental error since every precaution was taken to prevent contamination of the material worked with. The difficulty of securing results on ice cream, because of its physical condition, has already been pointed out and this factor is undoubtedly of importance here. It seems then that in general the hardening of ice cream results in a decrease in the bacterial content and that the decrease is quite variable in extent depending presumably on the hardening temperature and the resistance of the contained bacteria to the low temperatures encountered.

(g) *Influence of softening and rehardening on the bacterial count*

The effect of softening on the bacterial content of ice cream is a point of considerable importance, since, under the conditions of holding in retail establishments, softening occasionally occurs. There are two possible influences on the bacteria in ice cream as the result of softening and rehardening; the increase in the temperature with the melting of some of the material may allow of the growth of certain of the bacteria while the subsequent hardening may cause the destruction of certain organisms as in the case of the original hardening. It would be expected that the factor which would be of the most importance would depend on the types of bacteria present.

The data secured during the study of the influence of softening on the bacterial content of ice cream are presented in tables LVIII and LIX; the data are divided into two tables because more definite information regarding the temperatures reached was secured on the samples presented in table LIX than on those presented in table LVIII. Before discussing the results of the experiments, it should be pointed out that with the softening of the ice cream there is a tendency for the lighter material to come to the top and from a small amount of work done in this laboratory, it seems that the fat rising in softened ice cream carried bacteria with it just as fat rising in milk does. This means that the experimental error is greater here than it would be in ice cream kept properly hardened.

The data presented in table LVIII show an increase in all cases after softening and rehardening but in a number of instances the increases were so small as to be within the limit of experimental error. In certain of the tests, however, there were increases which are so large that there was very evidently an increase in the number of organisms contained.

In table LIX there was an increase in the number of bacteria on softening and rehardening in six cases and a decrease in eight cases. Generally, altho by no means constantly, the de-

TABLE LVIII. EFFECT OF SOFTENING AND REHARDENING OF ICE CREAM ON THE BACTERIAL CONTENT

Bacteria per c. c. before softening	Bacteria per c. c. after rehardening	Conditions of softening
310,000	368,000,000	Softened by not icing on one day; then rehardened.
2,170,000	6,450,000	Only slightly softened.
3,675,000	4,300,000	Softened by not icing on one day; then rehardened.
156,500	11,450,000	Softened by not icing on one day; then rehardened.
1,170,000	2,160,000	Softened by not icing on one day; then rehardened.
149,000	320,000	Softened by not icing on one day; then rehardened.
131,000	150,000	Softened by not icing on one day; then rehardened.
1,400,000	1,750,000	Softened by not icing on one day; then rehardened.
2,150,000	3,150,000	Softened by not icing on one day; then rehardened.
1,875,000	3,550,000	Softened by not icing on one day; then rehardened.
1,500,000	3,650,000	Softened by not icing on one day; then rehardened.
2,650,000	4,050,000	Softened by not icing on one day; then rehardened.
34,000	71,500	Left 20 hrs. with little salt. Then salted for 6 hrs. to harden.
40,000	77,500	Left 20 hrs. with little salt. Then salted for 6 hrs. to harden.
35,500	59,500	Left 20 hrs. with little salt. Then salted for 6 hrs. to harden.
42,500	66,000	Left 20 hrs. with little salt. Then salted for 6 hrs. to harden.
30,500,000	75,500,000	Lightly salted for 24 hrs.
23,000,000	91,500,000	Lightly salted for 24 hrs.
31,000,000	79,500,000	Lightly salted for 24 hrs.
28,500,000	80,000,000	Lightly salted for 24 hrs.

TABLE LIX. EFFECT OF SOFTENING AND REHARDENING OF ICE CREAM ON THE BACTERIAL CONTENT

Bacteria per c. c. before softening	Bacteria per c. c. after rehardening	Conditions of softening
2,300,000	2,950,000	Salted lightly and temp. up to 26.5°F; when rehardened only very slightly icy.
2,200,000	4,550,000	Salted lightly and temp. up to 26.5°F; when rehardened only very slightly icy.
360,000	330,000	Salted lightly and temp. up to 28.5°F; when rehardened very icy and volume considerably decreased.
350,000	315,000	Salted lightly and temp. up to 28.5°F; when rehardened very icy and volume considerably decreased.
220,000	400,000	Salted lightly and temp. up to 28.5°F; when rehardened slightly icy at top and very icy at bottom. Rancid flavor.
395,000	275,000	Salted lightly and temp. up to 28.5°F; when rehardened somewhat icy.
305,000	270,000	Salted lightly and softened for 2 days; temp. up to 32°F; when rehardened too icy to be marketable.
470,000	320,000	Salted lightly and softened for 2 days; temp. up to 32°F; when rehardened had settled a little and was too icy to be marketable.
26,000	18,000	Salted lightly and softened for 2 days; temp. up to 30°F; when rehardened was too icy to be marketable and the volume was decreased.
23,000	51,000	Salted lightly and softened for 2 days; temp. up to 30°F; when rehardened was too icy to be marketable.
160,000	480,000	Salted lightly and softened; temp. up to 26.5°F.
150,000	410,000	Salted lightly and softened; temp. up to 26.5°F.
1,500,000	1,100,000	Salted lightly and softened; temp. up to 32°F. When rehardened was too icy to be marketable.
1,745,000	1,070,000	Salted lightly and softened; temp. up to 32°F. When rehardened was too icy to be marketable.

creases occurred in the ice cream which reached the higher temperature during the softening and in which it would seem the rehardening would be the more destructive. The four samples which were allowed to reach a temperature of 32° F. during the softening period each showed a decrease when examined after the rehardening. Two samples were allowed to reach a temperature of 30° F. and one of these showed an increase after rehardening. Of the four samples which reached 28.5° F. three showed a decrease and one an increase and of the four samples which reached only 26.5° F. each showed an increase. From the extent of the softening allowed, these last four samples most nearly approximated the conditions existing with the samples reported in table LVIII and in which results similar to those secured on these four were obtained.

From the data presented in tables LVIII and LIX it seems that softening and rehardening may result in a very significant increase in the number of bacteria in ice cream or it may result in a decrease. An increase is more likely to occur in ice cream which is only slightly softened and then rehardened, while a decrease more commonly occurs in ice cream which is allowed to become very soft before rehardening: the decrease is undoubtedly due to a more destructive action of the rehardening after the ice cream has been allowed to reach the higher temperatures. In all probability the types of bacteria present are also very important in determining the nature and extent of the change in numbers that occurs. From table LIX it will be noticed that much of the ice cream allowed to reach the higher temperature was so icy on rehardening that it was unmarketable.

Summary

Within the last few years considerable attention has been given by boards of health to the establishment of sanitary standards for the ice cream sold within their jurisdictions. It is evident to anyone considering the matter that our knowledge of the bacteria in ice cream is insufficient to permit of the establishment of fair and logical bacterial standards. For this reason the work already reported from this station on the bacteriology of ice cream has been continued.

The importance of the contamination from the freezer was determined by adding sterile water to the freezer after which the machine was operated for approximately the freezing period. The number of bacteria per c.c. of water was then determined; in five trials the numbers of bacteria per c.c. ranged from 300 to 141,500 per c.c. It is probable that the contamination per c.c. of ice cream would have been considerably greater than the values given because of the fact that water fails to retain the air beaten into it and accordingly it was necessary to use a larger

volume of water than would have been used had an ice cream mix been added. These results indicate that the freezer may be an important source of contamination. Where an effort is being made to produce ice cream with a low bacterial count, the inadvisability of allowing the freezer to stand undried is quite evident because under these conditions there may be a considerable increase in the number of organisms in the water present. In all probability the proper care of the freezer demands the sterilizing of the freezing chamber just before the machine is used.

The numbers of bacteria in seventeen samples of water sherbet were determined. The counts ranged from 6 to 7,800 per c.c. and there was evidently no relationship between the flavor of the sherbet and the number of bacteria. The small numbers of bacteria found in water sherbets were undoubtedly due to the fact that no milk or cream was used in their manufacture. This idea is in agreement with the suggestion that the most important source of the bacteria in ice cream is the cream.

The numbers of bacteria in thirteen samples of ice cream other than vanilla were determined. The counts ranged from 130,000 to 40,850,000 per c.c. From these results it is evident that, from a bacteriological standpoint, ice cream other than vanilla presents the same problems as does ordinary ice cream.

Altho this station had previously published results on the changes in the number of organisms during the storage of ice cream, the importance of this point demanded the examination of additional samples. Accordingly thirty-nine samples of ice cream were studied during their storage with ice and salt and twelve samples were studied during their retention in a commercial hardening room. From the results obtained, it is quite evident that under ordinary conditions, as long as the product is kept properly hardened, there is no appreciable increase in the number of organisms in ice cream and that commonly there is a decrease. These results apply to the organisms developing on agar held at 37° C. for forty-eight hours.

The influence of the freezing process was studied in 51 cases by determining first the number of bacteria in the ice cream mix and then the number in the frozen material as it ran from the freezer. In two cases (4%) there was no change in the numbers, in 6 cases (11.8%) there was a decrease during the freezing process varying from 2 to 31% and averaging 13.0%, while in 43 cases (84.3%) there was an apparent increase varying from 2 to 227% and averaging 46.3%. From these results it is evident that in general there is an apparent increase in the numbers of organisms as determined by the plate method during the freezing of ice cream. The big factor in accounting for this increase in the number of organisms is, in all probability, the

breaking up of the clumps of organisms as a result of the violent agitation.

The influence of hardening on the bacterial content of ice cream was determined in 52 comparisons by securing the bacterial content of the ice cream as it ran from the freezer and later securing the bacterial content after the material had been suitably hardened; in one case (2%) there was no change in numbers, in 45 cases (86.5%) there was a decrease during the hardening process varying from 2 to 75% and averaging 39.1%, while in six cases (11.5%) there was an increase varying from 7 to 22% and averaging 13.8%. From these results it is evident that there is, in general, a decrease in the numbers of bacteria during the hardening of ice cream. The decrease is in all probability due to the destructive action of the lowered temperature.

The effect of softening and rehardening ice cream on the bacterial content evidently is somewhat variable. Whether there will be a decrease or an increase is determined very largely by the types of organisms present and by the extent of the softening. An increase is more likely to occur in ice cream which is only slightly softened, while a decrease more commonly occurs in ice cream which is allowed to become very soft before rehardening; the decrease in the latter case is, in all probability, due to the more destructive action of the lowered temperature after the softening at the higher temperature.

Conclusions

1. The freezer may be an important source of contamination where an effort is being made to produce ice cream with a low bacterial count and accordingly considerable attention should be given its care.
2. Water sherbets contain but few bacteria compared to the number ordinarily found in ice cream. The counts on 17 samples ranged from 6 to 7,800 per c.c.
3. Ice creams other than vanilla ordinarily contain large numbers of bacteria. The counts on 13 samples ranged from 130,000 to 40,850,000 per c.c.
4. There is no evidence that there is an increase in the numbers of contained organisms during the proper storage of ice cream while commonly there is a decrease. These results apply to the organisms developing on agar held at 37° C. for 48 hours.
5. There is an apparent increase in the number of bacteria as determined by the plate method during the freezing of ice cream. This is apparently due to the breaking up of the clumps of organisms as a result of the agitation in the freezer.
6. There is usually a decrease in the number of bacteria in

ice cream during the hardening process, presumably as a consequence of the destructive action of the lowered temperatures.

7. The softening and rehardening of ice cream may result in a significant increase or in a decrease in the number of bacteria contained. The effect is probably dependent on the types of bacteria present and on the extent of the softening, a decrease being more likely to occur when the ice cream is softened at a higher temperature since under these conditions the rehardening has a more destructive action.

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